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### Abstract Authors

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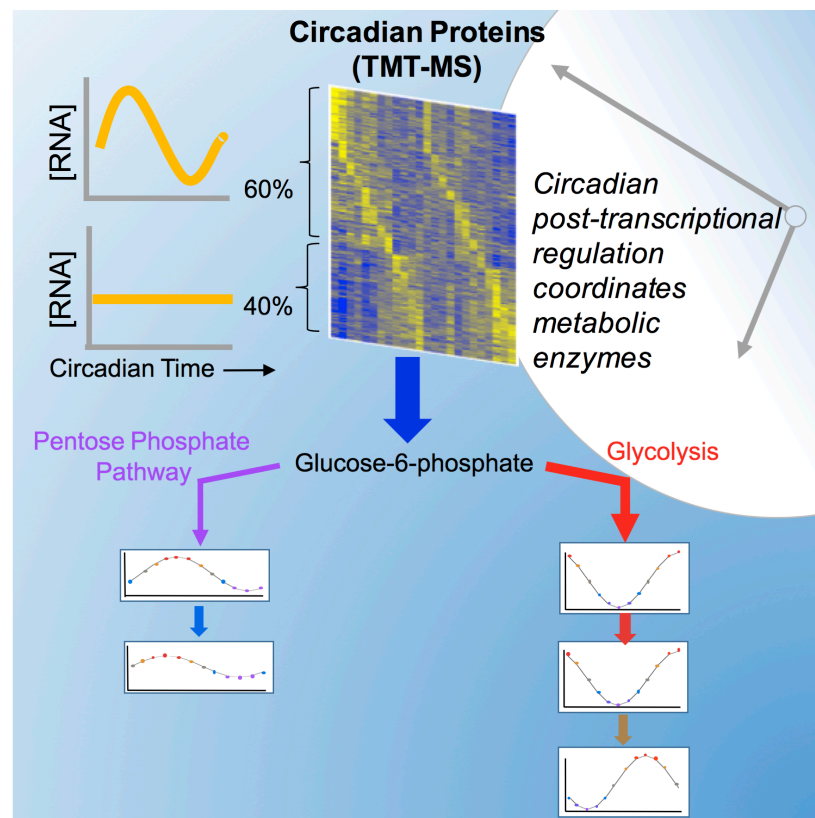
### Abstract Text

Nature selects those organisms that can reproduce the fastest while maintaining fitness and extracting the least amount of energy from their environment. This challenge makes regulation of natural systems critically important. We report the computational prediction of regulation, metabolite levels and rate constants using a maximum entropy method [1], and the experimental detection of circadian regulation of proteins and transcripts [2].

The computational method is applied in four steps: (1) a new constrained optimization approach based on Marcelin's 1910 mass action equation is used to obtain the maximum entropy distribution, (2) the predicted metabolite concentrations are compared to those generally expected from experiment using a loss function from which post-translational regulation of enzymes is inferred, (3) the system is re-optimized with the inferred regulation from which rate constants are determined from the metabolite

concentrations and reaction fluxes, and finally (4) a full ODE-based, mass action simulation with rate parameters and allosteric regulation is obtained. The method is applied to central metabolism and the flow of material through the three competing pathways of upper glycolysis, the non-oxidative pentose phosphate pathway, and the oxidative pentose phosphate pathway are evaluated as a function of the NADP/NADPH ratio.

The simulations complement experimental transcriptional and translational experiments (figure) performed over the circadian cycle of *Neurospora*. Transcriptional/translational feedback loops in fungi and animals drive circadian rhythms in



transcript levels that provide output from the clock, but post-transcriptional mechanisms also contribute. To determine the extent and underlying source of this regulation, we applied novel analytical tools to a long-duration, deeply-sampled, circadian proteomics time course comprising half of the proteome. We found a quarter of expressed proteins are clock-regulated, but >40% of these do not arise from clock-regulated transcripts. Contrary to predictions, rhythmic protein degradation plays little role in post-transcriptional regulation but instead rhythms arise from oscillations in translation. Our data highlighted the impact of the clock on metabolic regulation, with central carbon metabolism reflecting both transcriptional and post-transcriptional control and opposing metabolic pathways showing peak activities at different times of day. CSP-1, a transcription factor with a role in metabolic regulation of the clock, contributes significantly to determining the rhythmicity and phase of clock-regulated proteins. The experimental data demonstrate that the rhythmic proteins within the Pentose-Phosphate pathway peak in the circadian morning, while conversely, in glycolysis and the TCA cycle, the rhythmic proteins peak in the circadian evening. That is, the rhythmic proteins of glycolysis are in anti-phase to the rhythmic proteins of the Pentose-Phosphate pathway.

**10 Simple Rules:** The **general context** of the model is defined in all publications and in the Jupyter notebooks accompanying the model. Currently, the model only addresses the rate equations of metabolic processes, and only parameters for this **context** are available. In each publication and in the Jupyter notebooks, care is taken to describe how **metabolomics data** can **appropriately** be incorporated into the model, and the limits of the data itself. The model is **currently limited** to a well-mixed assumption regarding distribution of metabolites in the cellular compartments. **Version control** is maintained through Github repositories. The simulation method is **documented** not only in the methods section of each publication, but each modeling publication includes a Jupyter notebook demonstrating the implementation and use of the model. Each model is **disseminated** as a Jupyter notebook that includes not only the model, but the implementation of the model in code. This year, the model and approach was **evaluated (UQ)** by students from the University of Riverside Mathematics Department. We have now **implemented** various versions of general model in Matlab, Python and C. The results are implementation independent. Our current metabolic model is available in **SBML**, but not yet is the full simulation model available in SBML.

## References

1. Cannon, W.R., et al., *Prediction of metabolite concentrations, rate constants and post-translational regulation using maximum entropy-based simulations with application to central metabolism of Neurospora crassa*. Processes, 2018. 6(6)<https://doi.org/10.3390/pr6060063>
2. Hurley, J.M., et al., *Circadian Proteomic Analysis Uncovers Mechanisms of Post-Transcriptional Regulation in Metabolic Pathways*. Cell Systems, 2018<https://doi.org/10.1016/j.cels.2018.10.014>